PCT/EP 98/06749

Europäisches Patentamt

European Patent Office

Office européen des brevets

09/530209

REC'D 03 FEB 1999

WIPO PCT

Bescheinigung

Certificate

Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

97203303.9

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

M.B. RIJLING

DEN HAAG, DEN
THE HAGUE,
LA HAYE, LE

11/01/99

EPA/EPO/OEB Form 1014 - 02.91

Europäisches Patentamt | Eur pean Patent Office | Office eur péen des brevets

Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

Anmeldung Nr.:
Application no.: 97203303.9
Demande n°:

Anmeldetag:
Date of filing: 24/10/97
Date de dépôt:

Anmelder:
Applicant(s):
Demandeur(s):
CropDesign N.V.
9052 Zwijnaarde 3
BELGIUM

Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention:
A mitogenic cyclin of arabidopsis thaliana and the use in plant cell growth control

In Anspruch genommene Prioriät(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat: State: Pays:	Tag: Date: Date:	Aktenzeichen: File no. Numéro de dépôt:

Internationale Patentklassifikation:
International Patent classification:
Classification internationale des brevets:
C12N15/29, C07K14/415, C12N15/82, A01H5/00

Am Anmeldetag benannte Vertragstaaten:
Contracting states designated at date of filing: AT/BE/CH/[redacted]/DE/DK/ES/FI/FR/GB/GR/IE/IT/LI/LU/MC/NL/PT/SE
Etats contractants désignés lors du depôt:

Bemerkungen:
Remarks:
Remarques:

The application was transferred from the original applicant Vlaams Interuniversitair Instituut voor Biotechnologie vzw. to ~~the above-mentioned applicant on 09.09.98.~~

2

OAV2 / DI / CC2 / 001 - 009 / EP

A mitogenic cyclin of Arabidopsis thaliana and the use in plant cell growth control

D.G.Inze
L.O.M. De Veylder
J. De Almeida

The present invention relates to a new plant cyclin gene of *Arabidopsis thaliana*. The current invention concerns the isolation and purification of a nucleotide sequence and polypeptide sequence encoded by said nucleotide sequence.

Modulation of the expression of(a part of) the nucleotide sequence encoding a given polypeptide, alone or in combination with other sequences, has surprisingly an advantageous influence on plant cell division characteristics. Especially the identification of this gene provides a tool for study of the role of cyclins in plant cell cycle, growth and development. As a result thereof the total architecture, growth and sensitivity to the environment of the plant concerned can be manipulated by the regulation of the expression of the cyclin gene according to the invention.

The control of cell cycle progression in eukaryotes is mainly exerted at two transition points : one in late G_1, before DNA synthesis, and one at the G_2/M boundary. Progression through these control points is mediated by cyclin-dependent protein kinase (cdk) complexes, which contain a catalytic subunit of approximately 34-kDa encoded by the *cdk* genes. Cdk protein is active as a

protein kinase only when it is bound to a second protein called cyclin. Both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* only utilise one *cdk* gene for the regulation of their cell cycle. The kinase activity of their gene products $p34^{cdc2}$ and $p34^{cdc28}$ in *Sch. pombe* and in *S. cerevisiae*, respectively, is dependent on the regulatory proteins, so-called cyclins as mentioned above. Progression through the different cell cycle phases is achieved by the sequential association of $p34^{cdc2/cdc28}$ with different cyclins. Although in higher eukaryotes this regulation mechanism is conserved, the situation is more complex since they have evolved to use multiple Cdks to regulate the different stages of the cell cycle. In mammals, seven Cdks have been described defined as Cdk1 to Cdk7, each binding a specific subset of cyclins.

The activity of cdk/cyclin complexes is regulated at five levels: (i) transcription of the *cdk* and cyclin genes; (ii) association of specific cdks with their specific cyclin partner; (iii) phosphorylation/ dephosphorylation of the cdk and cyclin subunits; (iv) interaction with other regulatory proteins such as *suc1/cks1* homologues and cell cycle kinase inhibitors (CKI); and (v) cell cycle phase-dependent destruction of the cyclins.

In *Arabidopsis thaliana*, thusfar two *cdk* genes have been isolated, *cdc2aAt* and *cdc2bAt*, of which the gene products share 56% amino acid identity. Both Cdks are distinguished by several features. First, only *cdc2aAt* is able to complement yeast $p34^{cdc2/cdc28}$ mutants. Second, Cdc2aAt and Cdc2bAt bear different cyclin-binding motifs (PSTAIRE and PPTALRE, respectively), suggesting they may bind distinct types of cyclins. Third, although both *cdc2aAt* and *cdc2bAt* show the same spatial expression pattern, they exhibit a different cell cycle phase-specific regulation. The *cdc2aAt* gene is expressed constitutively throughout the whole cell cycle. In contrast, *cdc2bAt* mRNA levels oscillate, being most abundant during the S and G_2 phases.

In addition, multiple cyclins have been isolated from *Arabidopsis*. The majority displays the strongest sequence similarity with the animal A- or B-type class of cyclins, but also D-type cyclins have been identified. D-type cyclins are only distantly related to other cyclins. In mammals D-type cyclins act as growth sensors, with their expression depending more on extracellular cues than on the

cell's position in the cell cycle. Consequently D-type cyclins has been suggested to mediate mitogenic stimuli with the release from quiescence. This is believed to be regulated through the hyperphosphorylation of the retinoblastoma protein (Rb) by CDK/cyclin D complexes. Rb is a tumour suppressor protein which plays an important role in controlling the onset of cell division. In its hypophosphorylated form, Rb is complexed with E2F-type transcription factors which are known to promote expression of S phase-specific genes. Binding of Rb to E2Fs thereby prevents S phase induction. Phosphorylated Rb is unable to form complexes with E2F transcription factors and allows DNA synthesis. All D-type cyclins show a specific amino acid motif (LXCXE) permitting them to bind Rb. The *Arabidopsis* D-type cyclins (cycD1, cycD2, and cycD3) were isolated by their ability to rescue a yeast strain-deficient in its G1-cyclins (Soni *et al.*; The Plant Cell, 7, pag. 85-103, 1995). They all contain the Rb interacting motif, and were shown to be mitogen inducible.

To search for new gene products in plants interacting with cdk's and/or their interacting proteins, the two-hybrid screening (Fields et al., Nature, 340, pag. 245-246, 1989) was exploited whereby cdc2aAt as bait and a cDNA library of a cell suspension as prey are used. The library was made from a mixture mRNA from Arabidopsis thaliana cell suspensions harvested at various growing stages: early exponential, exponential, early stationary and stationary phase. A positive clone was identified. The clone was designated as LDV 59 and encodes a novel mitogenic cyclin.

Using above-mentioned two-hybrid screening technique, the isolation and characterisation was performed of a purified DNA sequence comprising a DNA sequence defined in SEQ.ID NO.1 encoding for a protein or part thereof or for a protein having substantially the same amino acid sequence as the protein defined in SEQ.ID.NO 2 respectively.

The *Arabidopsis thaliana* polypeptide according to the invention comprising the amino acid sequence as defined in SEQ.ID.NO 2 and/or a fragment thereof have a molecular weight of approximately 34 kDa.

Part of the invention is also a composition comprising the polypeptide according to SEQ.ID.NO. 2 and/or fragments thereof. An example for this is that a polypeptide or a fragment thereof according to the invention is embedded in another substantially purified amino acid sequence.

To the scope of the present invention also belong variants or homologues of amino acids enclosed in a polypeptide wherein said amino acids are substituted by other amino acids obvious for a person skilled in the art.

In addition the sequences as defined in any of the SEQ ID Nos 1 or 2 can be used to select homologous sequences present in other plants than Arabidopsis thaliana. Said selection preferably occur at the protein level using suitable hybridization techniques known to a skilled person.

Furthermore the invention concerns a method for manipulation plant cell growth comprising modulation of the expression of a gene encoding a polypeptide according to SEQ.ID.NO. 2 and/or fragments thereof.

Specifically the plant cell division rate and/or the inhibition of a plant cell division can be influenced by overexpression or reducing the expression of a gene encoding a protein according to the invention. Overexpression of a cyclin gene according to the invention promotes cell proliferation, while reducing cyclin expression arrests cell division. Part of the invention is thus the usage of a cyclin comprising the coding sequence or part thereof as mentioned in SEQ ID NO 1 as a negative or positive regulator of cell proliferation.

As a result of overproduction the G1/S generation time is shortened whereas the proliferation is less dependent on growth factors. A transformed plant can thus be obtained by transforming a plant cell with a gene encoding a polypeptide concerned or fragment thereof alone or in combination, whereas the plant cell may belong to a monocotyledonous or dicotyledonous plant. For this purpose tissue specific promoters, in one construct or being present as a separate construct in addition to the sequence concerned, can be used. Alternatively the expression of the cyclin is inducible by cytokinines or sucrose.

Surprisingly using a polypeptide or fragment thereof according to the invention or using antisense RNA for the gene according to the invention cell division of the meristems of the plant can be manipulated, positively and/or

negatively respectively. Furthermore, overproduction of the cyclin enhances growth and results in cell division to be less sensitive to an arrest caused by environmental stress such as salt, drought, chilling and the like. In addition part of the present invention is a method for transforming plants with a gene encoding for the amino acid sequence according to SEQ.ID.NO 2. Said transformation can for instance be accomplished by the so-called leaf disk protocol as described in Horsch et al, 1985,Science,277, p.1229-1231.

The present invention is also directed to a transgenic plant carrying a plant cell comprising a nucleotide sequence according to SEQ.ID.NO.1 with a controllable sequence adjacent thereto. This control mechanism includes introduction of high constitutive or inducible promoters adjacent to said nucleotide sequence or fragment thereof.

Part of the invention is also a plant cell carrying the coding nucleotide sequence as depicted in SEQ.ID.NO.1 wherein another than the naturally occurring promoter is located adjacent to said nucleotide sequence and/or fragment thereof.

Furthermore the purified DNA sequences as defined in SEQ ID NO 1 can be part of a recombinant expression vector operably linked to a suitable control sequence. Plant cells can be transfected or transduced accordingly with said recombinant expression vector.

In order to clarify what is meant in this description by some terms a further explanation is given hereunder.

The polypeptides of the present invention are not necessarily translated from a designated nucleic acid sequence; the polypeptides may be generated in any manner, including for example, chemical synthesis, or expression of a recombinant expression system, or isolation from virus. The polypeptides may include one or more analogs of amino acids or unnatural amino acids. Methods of inserting analogs of amino acids into a sequence are known in the art. The

polypeptides may also include one or more labels, which are known to those of skill in the art.

The terms "polynucleotide" ," nucleic acid sequence" or "nucleotide sequence" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double- and single-stranded DNA, and RNA. It also includes known types of modifications, for example, methylation, "caps" substitution of one or more of the naturally occuring nucleotides with an analog.

An "expression vector" is a construct that can transform a selected host cell and provides for expression of a heterologous coding sequence in the selected host. Expression vectors can be either cloning vectors or integrating vectors.

A "coding sequence" is a polynucleotide sequence which is transcribed into mRNA and/or translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to mRNA, cDNA, and recombinant polynucleotide sequences.

"Control sequence" refers to polynucleotide regulatory sequences which are necessary to effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, control sequences generally include promoter, ribosomal binding site, and terminators. In eukaryotes generally control sequences include promoters, terminators and, in some instances, enhancers. The term "control sequences" is intended to include, at a minimum, all components the presence of which are necessary for expression, and may also include additional advantageous components.

"Operably linked" refers to a juxtaposition wherein the components so ~~described are in a relationship permitting them to function in their intended~~

manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

"Polypeptide" refers to a polymer of amino acids and does not refer to a specific length of the molecule. Thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. This term also does not refer to or exclude post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

"Transformation" as used herein, refers to the insertion of an exogenous polynucleotide into a host cell, irrespective of the method used for the insertion, for example, direct uptake, transduction, or electroporation. The exogenous polynucleotide may be maintained as a non-integrated vector, for example, a plasmid, or alternatively, may be integrated into the host genome.

"Sense strand" refers to the strand of a double-stranded DNA molecule that is homologous to a mRNA transcript thereof. The "anti-sense strand" contains a sequence which is complementary to that of the "sense strand".

“Cell cycle” means the cyclic biochemical and structural events occuring during proliferation of cells. The cycle is divided into periods called: G_0, Gap_1 (G_1), DNA synthesis (S_1), Gap_2 (G_2), and mitosis (M).

“Cyclin” means one of the proteins which actively regulate cell division.

“Cell division” means mitosis, i.e. the usual process of cell reproduction.

"Cyclin-dependent protein kinase complex" means the complex formed when a cyclin associates with a cyclin dependent kinase. Such complexes may be active in phosphorylating proteins and may or may not contain additional protein species.

"Expression" means the production of a protein or nucleotide in the cell.

"Proliferation" means growth and reproduction, i.e. division of cells.

"Protein kinase" means an enzyme catalyzing the phosphorylation of proteins.

In the description of the current invention reference is made to the following sequences of the Sequence Listing:

SEQ.ID.NO. 1 : nucleotide sequence of the clone LDV 59.

SEQ.ID.NO. 2 : amino acid sequence obtainable from the coding nucleotide sequences represented in SEQ ID NO 1.

The present invention is further described by reference to the following non-limiting figures and examples.

Example 1

Two hybrid screen using Cdc2aAt as bait.

To identify Cdc2aAt-interacting proteins a two-hybrid system was used based upon GAL4 recognition sites to regulate the expression of both *his3* and *lacZ* reporter genes. The pGBTCDC2A vector, containing a fusion protein between the C-terminus of the GAL4 DNA-binding domain and Cdc2aAt was constructed by cloning the full-lenght coding region of *cdc2aAt* into the pGBT9 vector. For the screening a GAL4 activation domain cDNA fusion library was used, constructed from mRNA of *Arabidopsis thaliana* cell suspensions harvested at various growing

stages: early exponential, exponential, early stationary, and stationary phase. The pGBTCDC2A plasmid was cotransformed with the library into the HF7c reporter strain. Approximately 1.2 10^7 independent transformants were screened for their ability to grow on histidine-free medium. A 3-day incubation at 30°C yielded about 1200 colonies. These colonies were tested for their growth on medium without histidine in the presence of 10 mM 3-amino-1,2,4-triazole, reducing the number of positives to 250. Next these colonies were tested for the activation of the *lacZ* gene, and 153 turned out to be both His^+ and $LacZ^+$. DNA was prepared from the positive clones and sequenced. One of the clones contained a gene (LDV59) encoding a novel mitogenic cyclin. The LDV59 encoded protein also contains the Rb interacting motif.

The specificity of the interaction between the LDV59 encoded protein with Cdc2aAt was verified by the retransformation of yeast with pGBTCDC2A and pGADLDV59. As controls, pGBTCDC2A was cotransformed with a vector containing only the GAL4 activation domain (pGAD424); and pGADLDV59 was cotransformed with a plasmid containing only the GAL4 DNA-binding domain (pGBT9). Transformants were plated on medium with or without histidine. Only transformants containing both pGBTCDC2A and pGADLDV59 were able to grow in the absence of histidine.

Example 2

The LDV59 encoded protein associates with both Cdc2aAt and Cdc2bAt.

The pGBTCDC2B vector encoding a fusion protein between the C-terminus of the GAL4 DNA-binding domain and Cdc2bAt was constructed by cloning the full-lenght coding region of *cdc2bAt* into the pGBT9 vector. pGBTCDC2B was transformed with pGADLDV59 in the HF7c yeast and cotransformants were plated on medium with or without histidine. As controls, pGBTCDC2B was cotransformed with a vector containing only the GAL4 activation domain (pGAD424); and pGADLDV59 was cotransformed with a plasmid containing only the GAL4 DNA-binding domain (pGBT9). Only transformants containing both pGBTCDC2B and pGADLDV59 were

able to grow in the absence of histidine. These data demonstrate that the LDV59 encoded gene product not only binds to Cdc2aAt, but also to Cdc2bAt.

Example 3

Characterization of the LDV59 gene.

To study the genomic organization of the LDV59 gene, *Arabidopsis* DNA was digested with 3 different enzymes. Hybridization with the LDV59 coding region at low stringency showed only one band for every digest, indicating the presence of only one LDV59 gene per haploid genome of *Arabidopsis*.

Example 4

LDV59 expression is mitogenic inducible

Arabidopsis cell suspensions ecotype Col.-O, maintained as described by Glab et al. (FEBS Lett., 353, pag. 207-211) were depleted for growth factors for 48 hrs. by resuspending them in medium lacking auxin (2,4-D), cytokinin (BAP), and sucrose. After 48 hrs. the cells were split into eight aliquots, which were resuspended in medium containing sucrose or lacking sucrose, containing auxin or lacking auxin, and containing cytokinin or lacking cytokinin. After 6 hrs. cultivation RNA was extracted from the cells. An RNA gel blot was probed with the LDV59 gene. A hybridization signal was only observed for the cells supplemented with cytokinin and sucrose, indication that the LDV59 gene is specially induced by these mitogenic agents.

Example 5

LDV 59 expression

Plant material was fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH7.2). Fixed tissue was dehydrated with ethanol, cleared with toluene, and embedded in paraffin. Embedded tissue was sliced into serial 10μm sections and attached to coated microscope slides. ^{35}S-UTP-labeled sense and antisense RNA of a cDNA LDV59 subcloned in PGem2 were generated by run-off transcription using T7 and Sp6 RNA polymerases according to the manufacturer's instructions (Boehringer Mannheim). Full-length transcripts were reduced to an average length of 0.3kb by alkaline hydrolysis (Cox et al., 1984,Dev.Biol.,101,p.485). The size of both full-length and hydrolyzed transcripts was checked on a 1% denaturing gel, and the amount of synthesized RNA was calculated. The mRNA in situ hybridization procedure was carried out essentially as described by Angerer and Angerer (1992) in "In situ hybridization": A practical approach(The Practical Approach series, Wilkinson DG,ed,Oxford Press,pp.15-32). Stringent wash was performed in 0.1SSC (1X SSC; 150mM NaCl, 15mM Na3-citrate, pH7.0) at 62°C for 60 min to avoid cross hybridization.

By applying the mRNA *in situ* hybridization technique on roots, shoot apical meristems and flowers of Arabidopsis thaliana and on radish roots, the following expression patterns were observed.

Early, during lateral root development, pericycle cells neighbouring one protoxylem pole show an intense hybridization signal. As lateral roots expand very strong signal is mainly observed at its basis. At that stage a weak or no signal is detected in the meristematic cells of the lateral root tip. At later stages of root development, only a subset or group of cells in the root meristem show high accumulation of LDV59 mRNA. Mature root meristems barely show any expression of the LDV59 gene. Cell files in specific regions along the vascular tissue show a weak and uniform expression pattern. Similar cell files of the vascular tissue show a completely different pattern of expression. Alternating stretches of cells along the vascular cylinder expressing and not expressing the LDV59 are observed. Some tissue sections along the vascular tissue contained only one single cell expressing the LDV59 gene.

During flower development LDv59 expression was observed along the vascular tissue from the filament and young ovaries. At later stages, high accumulation of LDV59 mRNA was observed in the fertilized ovule, most likely in the megaspore-mother-cell burried in the nucellus tissue. During embryo development expression was high in globular and heart stages embryos and low in mature embryo. These results shows that LDV59 is involved in early steps of vascular tissue, lateral root formation and embryo development.

Sequence Listing of clone LDV 59

SEQ ID NO 1

ATG GCAGAGGAAA ATCTAGAACT GAGTCTTTTA TGTACAGAGA GCAACGTTGA TGATGAGGGC

ATGATTGTTG ACGAAACTCC GATTGAAATT TCGATTCCTC AGATGGGTTT TTCTCAATCG

GAGAGTGAGG AGATTATCAT GGAGATGGTG GAGAAGGAGA AGCAGCATTT GCCAAGTGAT

GATTACATCA AGAGACTTAG AAGTGGAGAT TTGGATTTGA ATGTTGGAAG AAGAGATGCC

CTCAATTGGA TTTGGAAGGC TTGTGAAGTA CACCAGTTTG GACCATTGTG TTTTTGCTTA

GCAATGAACT ACTTGGATCG ATTCTTATCG GTTCATGATT TGCCTAGTGG CAAAGGTTGG

ATATTGCAGT TGTTGGCTGT GGCTTGTTTA TCATTGGCAG CCAAAATTGA AGAAACTGAA

GTTCCAATGT TGATAGATCT TCAGGTTGGA GATCCTCAGT TTGTGTTTGA GGCTAAATCA

GTCCAAAGAA TGGAGCTTTT GGTGTTGAAC AAATTGAAAT GGAGATTGAG AGCAATAACT

CCATGCTCAT ACATAAGATA TTTCCTGAGA AAGATGAGTA AATGTGATCA AGAACCATCC

AACACATTGA TATCTAGATC ATTACAAGTG ATAGCCAGCA CAACCAAAGG TATTGACTTT

TTGGAGTTTA GACCTTCTGA AGCTGCTGCT GCTGTGGCAC TTTCTGTTTC TGGAGAATTG

CAGAGAGTAC ACTTTGACAA CTCTTCCTTC TCTCCTCTTT TCTCACTACT TCAAAAGGAG

AGAGTGAAGA AGATAGGGGA AATGATAGAG AGTGATGGCT CAGACTTATG TTCACAAACA

CCCAATGGGG TTTTAGAAGT ATCGGCTTGT TGTTTCAGCT TTAAGACCCA TGATTCTTCT

TCTTCTTATA CACATCTTTC TTAA

SEQ ID NO 2

MetAlaGluGlu AsnLeuGlu LeuSerLeuLeu CysThrGlu SerAsnVal AspAspGluGly

MetIleVal AspGluThr ProIleGluIle SerIlePro GlnMetGly PheSerGlnSer

GluSerGlu GluIleIle MetGluMetVal GluLysGlu LysGlnHis LeuProSerAsp

AspTyrIle LysArgLeu ArgSerGlyAsp LeuAspLeu AsnValGly ArgArgAspAla

LeuAsnTrp IleTrpLys AlaCysGluVal HisGlnPhe GlyProLeu CysPheCysLeu

AlaMetAsn TyrLeuAsp ArgPheLeuSer ValHisAsp LeuProSer GlyLysGlyTrp

IleLeuGln LeuLeuAla ValAlaCysLeu SerLeuAla AlaLysIle GluGluThrGlu

ValProMet LeuIleAsp LeuGlnValGly AspProGln PheValPhe GluAlaLysSer

ValGlnArg MetGluLeu LeuValLeuAsn LysLeuLys TrpArgLeu ArgAlaIleThr

ProCysSer TyrIleArg TyrPheLeuArg LysMetSer LysCysAsp GlnGluProSer

AsnThrLeu IleSerArg SerLeuGlnVal IleAlaSer ThrThrLys GlyIleAspPhe

LeuGluPhe ArgProSer GluAlaAlaAla AlaValAla LeuSerVal SerGlyGluLeu

GlnArgVal HisPheAsp AsnSerSerPhe SerProLeu PheSerLeu LeuGlnLysGlu

ArgValLys LysIleGly GluMetIleGlu SerAspGly SerAspLeu CysSerGlnThr

ProAsnGly ValLeuGlu ValSerAlaCys CysPheSer PheLysThr HisAspSerSer

SerSerTyr ThrHisLeu Ser

Claims

1. Plant cell cycle interacting proteins obtainable by a two-hybrid screening assay whereby cdc2aAt as bait and a cDNA library of a cell suspension as prey are used.

2. A purified DNA sequence comprising a DNA sequence defined in SEQ.ID.NO.1 encoding for a protein or part thereof or encoding for a protein having substantially the same amino acid sequence as the protein defined in SEQ.ID.NO 2.

3. An Arabidopsis thaliana polypeptide comprising an amino acid sequence according to SEQ.ID.NO. 2 and/or fragments thereof.

4. Composition comprising a polypeptide according to SEQ.ID.NO.2 and/or fragments thereof.

5. A method for manipulation plant cell growth comprising modulation of the expression of a gene encoding a polypeptide and/or fragment thereof according to claim 2 or 3.

6. A method to influence a plant cell division rate and/or inhibition of a plant cell division by overexpression or reducing the expression of a gene encoding a polypeptide defined in SEQ.ID.NO. 2 and/or fragments thereof.

7. A method according to claims 5 or 6 wherein the plant cell belongs to a monocotyledonous or dicotyledonous plant.

8. A plant cell carrying a nucleotide sequence according to claim 2.

9. A plant cell carrying a nucleotide sequence according to SEQ.ID.NO. 1 wherein another than the naturally occurring promoter is located adjacent to said nucleotide sequence and/or fragment thereof.

10. A plant cell carrying a substantially purified polypeptide with the amino acid sequence according to SEQ.ID.NO. 2 and/or fragments thereof.

11. A method for transforming plants with a gene encoding for the amino acid sequence according to SEQ.ID.NO. 2 and/or fragments thereof.

12. Transgenic plant comprising a plant cell according to claim 8, 9 or 10.

13. A recombinant expression vector comprising a purified DNA sequence according to claim 2 operably linked to a suitable control sequence.

14. Plant cells transfected or transduced with a recombinant expression vector according to claim 13.

15. A process for producing a transgenic plant comprising the steps of introducing a purified DNA sequence according to claim 2 into plant cells and regenerating a plant from said cells.

16. A process for inhibiting plant cell division by reducing the expression of a nucleotide sequence as mentioned in SEQ ID NO 1 in said plant cell.

17. Use of a cyclin comprising the coding sequence or part thereof as mentioned in SEQ ID NO 1 as a positive or negative regulator of cell proliferation.

18. Use of a sequence mentioned in SEQ ID NO 1 and/or SEQ ID NO 2 to select homologous sequences present in other plants than Arabidopsis thaliana.

19. A method to modify the growth inhibition of plants caused by environmental stress conditions by using any of the SEQ ID NO 1 and/ or SEQ ID NO 2 where in addition optionally another than the naturally occuring promoter is located adjacent to the sequence.

4

Abstract

The present invention relates to a new plant cyclin gene of *Arabidopsis thaliana.* And concerns the isolation and purification of a nucleotide sequence and polypeptide sequence encoded by said nucleotide sequence.

Modulation of the expression of(a part of) the nucleotide sequence encoding a given polypeptide, alone or in combination with other sequences, has surprisingly an advantageous influence on plant cell division characteristics. Especially the identification of this gene provides a tool for study of the role of cyclins in plant cell cycle, growth and development. As a result thereof the total architecture, growth and sensitivity to the environment of the plant concerned can be manipulated by the regulation of the expression of the cyclin gene according to the invention.